

ZINC, SURGICAL TRAUMA AND REPAIR

by

Ingrid Tengrup

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Ingrid Tengrup

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Abstract Serum zinc concentrations were registered in 57 patients before, during and after surgical trauma of different magnitude. A marked decrease in serum zinc occurred after major trauma with maximum 6 hours postoperatively. Zinc supplementation for 4 weeks prior to operation in 8 patients resulted in increased serum zinc concentrations. The postoperative drop in serum zinc was of the same magnitude as in non-supplemented patients but the lowest values 6 hours postoperatively remained within the normal range of serum zinc. Experimental studies in rats were initiated to study the effect of zinc supplementation on granulation tissue formation. Zinc supplementation was given as intramuscular injections of zinc acetate solution. To obtain granulation tissue 6 polyvinyl sponges were implanted subcutaneously on the backs of the rats. The sponges were removed after 1-42 days of implantation, homogenated and analysed chemically. Some sponges were examined histologically and histochemically. Zinc supplementation to normal animals resulted in increased collagen amounts after 4 days of implantation compared with controls. Collagen synthesis was not influenced by zinc supplementation. Indirect evidence was found that decreased collagen breakdown in granulation tissue occurred after zinc supplementation. A more rapid decrease in alkaline phosphatase activity, localized in PMN leucocytes indicates a more rapid disappearance of PMN leucocytes from granulation tissue after zinc supplementation. As the PMN leucocytes are rich in collagenolytic enzymes this finding may be the explanation for the decrease in collagen breakdown after zinc supplementation.			
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Ingrid Tengrup

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Ingrid Tengrup

**From the Departments of Surgery, Experimental
Research and Nuclear Medicine, Malmö General Hospital,
University of Lund, Malmö, Sweden**



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AND REPAIR



The present dissertation is based on the following publications, which will be referred to in the text by their Roman numerals:

- I CHANGES IN SERUM ZINC DURING AND AFTER SURGICAL PROCEDURES
 In collaboration with H. Samuelsson
 Acta Chir Scand 143: 195-199, 1977
- II SERUM ZINC BEFORE AND AFTER CHOLECYSTECTOMY IN ZINC-TREATED
 PATIENTS
 In collaboration with B. Zederfeldt
 Acta Chir Scand 145: 293-295, 1979
- III GRANULATION TISSUE FORMATION IN ZINC-TREATED RATS
 In collaboration with J. Ahonen and B. Zederfeldt
 Acta Chir Scand 146: 1-4, 1980
- IV CYTOCHEMICAL STUDY OF GRANULATION TISSUE IN ZINC TREATED RATS
 In collaboration with J. Ahonen, F. Rank and B. Zederfeldt
 In press. Acta Chir Scand, 1980
- V INFLUENCE OF ZINC ON SYNTHESIS AND ACCUMULATION OF COLLAGEN
 IN EARLY GRANULATION TISSUE
 In collaboration with J. Ahonen and B. Zederfeldt
 In press. Surg Gynecol Obstet, 1980

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INTRODUCTION

Zinc is one of the so called trace elements in the human body which contains altogether approximately 2.0 g of zinc (Widdowson et al., 1951). Zinc is found in all human tissues and especially liver, kidney, prostate, skin and muscle contain high amounts of zinc (Tipton & Cook, 1963). In plasma zinc is bound mainly to albumin but also to α -2-macroglobulin, transferrin and ceruloplasmin. Between 2 and 3% of plasma zinc exists as an ultrafiltrable fraction partly in ionic form, partly bound to aminoacids (Giroux et al., 1976).

The major role of zinc in man and animals appears to be related to enzyme function. Zinc forms an integral part of many enzymes (Riordan, 1976). Zinc enzymes have been subdivided on the basis of their affinity for zinc into two groups: zinc enzyme complexes and zinc metallo-enzymes. There are now over 70 metallo-enzymes known to require zinc for their function.

Zinc metallo-enzymes bind zinc firmly with a considerable degree of specificity. At least two distinct functional roles of zinc in enzymes can be defined (Riordan, 1976): Maintenance of the structural integrity of the enzyme protein and direct participation in the progress of enzyme catalysis. Both functions can be found within a single enzyme.

Zinc absorption from the gut is variable and depends upon a variety of factors, e.g. the level of zinc in the diet (Spencer et al., 1974), the presence in the diet of substances potentially interfering with resorption (O'Dell & Savage, 1960; Sandström et al., 1978) and the zinc status in the body (Hallmans & Wing, 1979). Zinc is most readily absorbed from proteins in fish and meat.

Under normal conditions zinc is lost from the body in urine (approximately 0.5 mg per day), sweat (approximately 0.5 mg per day) and gastrointestinal tract (approximately 10-15 mg per day) (Spencer et al., 1974; Prasad et al., 1963a).

Zinc deficiency can be due to inadequate zinc supply in the diet, to impaired absorption from the gastrointestinal tract or to increased losses of zinc from the body.

Extreme zinc deficiency in man has been discovered in certain areas in Iran and Egypt (Prasad, 1979). The main cause for this deficiency is high dietary content of phytates, interfering with zinc absorption. Additional factors are low intake of animal proteins and zinc loss by sweat and from the gastrointestinal tract because of parasitic infections.

Moderate zinc deficiency depending on insufficient availability of zinc can be seen after long-term intravenous nutrition (Kay et al., 1976). Inadequate diet may be the explanation for the low serum zinc found by Hallböök & Hedelin (1977) in surgical patients more than 60 years of age. In acrodermatitis enteropathica low serum zinc levels are seen (Michaëlsson, 1974; Hambidge et al., 1978). The underlying mechanism is thought to be a defective zinc absorption from the gut. Low serum zinc levels are also seen in patients with liver cirrhosis due to alcohol abuse (Vallee et al., 1956). The patients have low serum and liver zinc but high urinary excretion of zinc (Vallee et al., 1957).

The clinical recognition of zinc deficiency in man (Prasad et al., 1963b) led to growing awareness of the essentiality of zinc for man. Clinical manifestations of zinc deficiency were growth retardation and sexual immaturity. Parakeratotic skin lesions have

been described in patients suffering from acrodermatitis enteropathica (Michaëlsson, 1974) and after long-term intravenous nutrition (Kay et al., 1976).

For over 100 years it has been known that zinc is essential for the growth of microorganisms (Raulin, 1869). It was, however, not until 1934 that zinc was shown to be necessary for the normal growth of higher organisms. The first animal examined was the rat (Todd et al., 1934). Since the demonstration by Tucker & Salmon in 1955 of the importance of zinc for the growth of farm animals, the use of zinc supplemented diets in the breeding industry has been widespread.

Manifestations of zinc deficiency in animals include growth retardation, poor appetite and parakeratotic skin lesions. Food intake is greatly increased in zinc deficient rats after zinc supplementation (Miller, 1970).

Acute reduction in serum zinc has been reported in connection with trauma and infections. The mechanism responsible for the decrease of serum zinc following stress is not completely clarified but increased tissue uptake of zinc particularly in the liver (Pekarek et al., 1972; Hallmans & Wing, 1979) increased urinary excretion of zinc (Sefton et al., 1974) and accumulation of zinc in the area of injury (Savlov et al., 1962) have been implicated. It may well be that all of these mechanisms contribute to the decrease of serum zinc concentration following stress.

As one of the most important effects of zinc seems to be maintenance of normal growth, it seems reasonable to assume that zinc can be of importance for tissue regeneration. Chronic zinc deficiency has been shown to impair wound healing in animals. Thus,

Sandstead & Shepard (1968), Sandstead et al. (1970) and Rahmat et al. (1974) have shown that the rate of closure of defects in the integument is decreased and the development of tensile strength of skin incisions are delayed in zinc deficient rats. Further, Sandstead et al. (1970) found that zinc supplementation has no effect on healing rate in normal rats.

Improved wound healing following zinc supplementation has been reported by Pories et al. (1967). In their investigation no registration of serum zinc concentration was reported. Other investigators (Barcia, 1970; Myers & Cherry, 1970) found no effect of zinc supplementation in patients with chronic pilonidal disease and chronic leg ulcers. Hallbökk & Lanner (1972) have shown that accelerated healing of chronic leg ulcers can be obtained by zinc supplementation but only in patients with initially low serum zinc values. It may well be that zinc supplementation is of value for wound healing only in subjects with low serum zinc values.

Thus, the literature contains evidence that zinc supplementation may promote wound healing in zinc deficient patients and animals.

The functional importance of acute decrease in serum zinc is not clear. Larson et al. (1970) have found low plasma zinc values in burned children returning to normal only when most of the wounds were covered. Zinc supplementation resulted in normal plasma zinc levels despite remaining open wounds. A tendency to more rapid healing with zinc supplementation was reported. The observations made in that investigation indicate that an acute lowering of serum zinc in connection with trauma may influence wound healing.

The purpose of the present investigation was to study zinc metabolism in connection with surgical trauma and its relation to wound healing. We have tried to elucidate the following problems:

- Changes in serum zinc following surgical trauma of different magnitude.
- Influence of preoperative zinc supplementation on alterations in serum zinc in connection with surgical trauma.
- Influence of zinc supplementation before and after trauma on the formation of granulation tissue in rats.
- Influence of zinc supplementation before and after trauma on the cell composition of early granulation tissue in rats.
- Influence of zinc supplementation on the collagen metabolism in early granulation tissue in rats.

CLINICAL INVESTIGATION OF SERUM ZINC CHANGES AFTER OPERATIVE TRAUMA (I, II)

Serum zinc concentration was followed in 57 patients of both sexes subjected to anesthesia alone or with different surgical procedures including hernia repair, cholecystectomy and highly selective vagotomy (HSV). Eight female patients were given 220 mg of zinc 3 times daily for 4 weeks prior to cholecystectomy (II).

Serum zinc concentrations were determined on admission to the hospital, after anesthesia but before surgery was started, immediately postoperatively and 6 hours after the conclusion of the

operation. Further, serum zinc concentrations were followed every morning the first 2-4 postoperative days. In the patients given preoperative zinc, serum zinc was also determined before the zinc supplementation was started.

Serum albumin was determined preoperatively and on the first postoperative day.

Venous blood was drawn with disposable needles into acid-washed glass tubes provided with acid-washed plastic stoppers. Only samples visibly free of hemolysis were examined. Serum zinc concentration was determined with atomic absorption technique using the method described by Parker et al. (1967). Normal range of serum zinc in our laboratory is 10-16 $\mu\text{mol/l}$, mean value 13 $\mu\text{mol/l}$.

Results

Serum zinc concentrations on admission to hospital in the first study and before zinc treatment in the second study were found to be within the normal range. Zinc administration for 4 weeks resulted in increased serum zinc concentrations, significantly above normal range.

Mean serum zinc concentration after anesthesia but before surgery was found to be about 10% below the preoperative zinc level in all groups. This difference is not statistically significant.

No differences were seen between spinal anesthesia and general anesthesia with the same surgical trauma.

Minor operative trauma as hernia repair was not followed by any changes in serum zinc concentration.

Cholecystectomy and HSV were followed by a marked decrease in serum zinc concentration with the lowest value 6 hours postoperatively.

Older patients tend to have a more pronounced decrease 6 hours postoperatively than do younger ones.

The decrease in serum zinc concentration was of the same magnitude in patients with preoperative zinc supplementation but due to the higher preoperative serum zinc values, the lowest value 6 hours postoperatively fell within the normal range of serum zinc concentration.

Serum zinc concentration returned to normal on the third to the fourth postoperative day and was at that time slightly above normal range in the patients supplemented preoperatively with zinc.

No changes in serum albumin were registered in any group.

Healing was without complications in all patients but one. This patient got a minor wound infection which did not influence the serum zinc concentration further.

Comments on clinical investigation

Fodor et al. (1973) have reported that general anesthesia leads to decrease of serum zinc concentration. This observation has not been confirmed in the present study, although there is a tendency to decrease of serum zinc concentration between the values preoperatively and after induction of anesthesia. If this decrease of serum zinc concentration is an effect of anesthesia or is connected to the albumin changes following bedrest cannot be evaluated (Jeppsson, 1977).

The findings correspond with the observations reported by Sefton et al. (1974) that major trauma causes reduction of serum zinc concentration. The maximum decrease in serum zinc concentration occurs 6 hours after the conclusion of the operation and nor-

mal values are restored within 3-4 days. Preoperative zinc supplementation leads to supernormal serum zinc concentration. The magnitude of the decrease in serum zinc postoperatively is not altered but due to high preoperative values the lowest postoperative values remain within the normal range.

Investigations on patients thus confirm that trauma causes decrease of serum zinc concentration. Such investigations do not allow detailed studies on granulation tissue formation. Therefore experimental investigations were initiated to elucidate if zinc supplementation in connection with trauma does influence granulation tissue formation.

EXPERIMENTAL INVESTIGATION (III, IV, V)

Material and Methods

Animals

Two hundred and forty-two white female Sprague-Dawley rats, weighing 180-200 g, were used. They were fed a pellet diet (Astra-Ewos, 100 ppm zinc) and were given tap water ad libitum.

Zinc supplementation

Fifty μ g zinc was administered intramuscularly daily as zinc acetate solution. The first dose of zinc was given half an hour before operation in most of the animals but in 20 animals zinc administration was started on the first postoperative day. The control animals had no zinc injections.

Anesthesia

Operations were performed under general anesthesia by mebumal natrium (45 mg/kg body weight) injected intraperitoneally.

Experimental procedure

To obtain granulation tissue 6 polyvinyl sponges, weighing 55-60 mg each, were implanted subcutaneously on the back of each animal according to Viljanto & Kulonen (1962) as modified by Pallin et al. (1975). The sponges were removed under ether anesthesia after 1-42 days of implantation and taken for analysis.

After removal the sponges were cut into small pieces and thereafter homogenized. The homogenates were analysed chemically.

Concomitant with sponge removal blood was obtained by heart puncture for the determination of serum zinc by day 4-42. The blood was drawn into acid-washed glass tubes provided with acid-washed plastic stoppers.

Chemical analysis

Serum zinc was determined with atomic absorption technique (Helwig et al., 1966; Michaëlson et al., 1976).

Hemoglobin concentration in sponge homogenates was determined after 4, 7, 14 and 42 days of implantation as cyanmethemoglobin as recommended by the International Committee for Standardization in Haematology (1965).

The activities in sponge homogenates of *alkaline phosphatase*, *acid phosphatase* and β -*glucuronidase* were determined after 1, 2, 3 and 4 days of implantation (Bessey et al., 1946; Andersch & Szczypinski,

1947; Fishman & Lerner, 1953; Talalay et al., 1946.

Collagen content in the sponge homogenates was determined according to Pikkarainen (1968) after 4, 7, 14 and 42 days of implantation as hydroxyproline.

Collagen synthesis was studied by *in vivo* incorporation of ^{14}C -L-proline into hydroxyproline after 3, 4 and 5 days of sponge implantation. Twenty-four hours before sponge removal 2.78 MBq ^{14}C -L-proline (10.730 MBq/mmol, manufactured by NEN Chemicals GmbH West Germany) was injected intravenously. Total radioactivity and activity of ^{14}C -hydroxyproline were determined according to Juva & Prockop (1966). Radioactivity was measured in a liquid scintillation counter (LKB Wallac 81000). At least 1000 cpm over background were registered.

The results were expressed as dpm/granuloma.

Collagen synthesis was also studied *in vitro* using slices from sponges removed after 4 days of implantation. The sponge slices were incubated in Krebs-Ringer solution with 0.37 MBq ^{14}C -L-proline for 150 minutes (Mobacken, 1974). Total radioactivity and the radioactivity of ^{14}C -hydroxyproline were determined as described above.

Hydroxyproline was determined according to Pikkarainen (1968) and was used for calculating specific activity of ^{14}C -hydroxyproline (dpm/ μmol hypro).

Histological and histochemical preparations

Histological and histochemical preparations were made on sponges implanted 1, 2, 3 and 4 days.

The sponges used for microscopical examinations were divided into two halves. One half was prepared by routine histological techniques and stained with hematoxylin-eosin and van Gieson. The other half was rapidly frozen in propane cooled by liquid nitrogen and stored at -70° C. Sections about 5 microns thick were processed in a cryostat. The azo-dye technique was used to demonstrate:

- Alkaline phosphatase with naphtol AS-TR phosphatase as substrate and Fast Red TR as diazonium salt (Aronsen et al., 1968).
- Acid phosphatase with naphtol AS-TR phosphoric acid as substrate (Barka & Anderson, 1962) and
- β -glucuronidase with naphtol AS-BT β -D-glucosiduronic acid as substrate (Hayashi et al., 1964).

"Hexazolised" fuchsin (pararosanilin) was used as diazonium salt (Barka & Anderson, 1962).

Normal rat liver was used as control for staining technique.

Statistical methods

In the statistical analysis of results the mean (M), the standard deviation (SD) and the standard error of the mean (SEM) were calculated. Comparisons between groups were carried out using either Student's t-test for unpaired observations or Mann-Whitney rank sum test. Differences were considered significant when $p < 0.05$.

Results

Collagen content in granulation tissue (III)

The amount of hydroxyproline in the sponges increases during the observation time in all groups. Zinc supplementation, starting preoperatively and continued through the period of study, results in higher amounts of hydroxyproline at day 4 whereas zinc given merely postoperatively has no effect on the amount of hydroxyproline.

The amount of hemoglobin in the sponges decreases from day 4 to day 7, is higher by day 14 and decreases again by day 42 in all animals. Preoperative zinc administration results in lower amounts of hemoglobin by day 4 compared with the control animals.

Serum zinc concentration is the same in control and zinc supplemented animals.

Collagen synthesis in early granulation tissue (V)

The radioactivity of hydroxyproline in sponges is low at day 3, higher at day 4 and even higher at day 5.

The total radioactivity is the same in sponges removed at day 3 and day 4 but higher in sponges removed at day 5.

The radioactivity of hydroxyproline is the same in control and zinc supplemented animals.

The amount of hydroxyproline in the sponges is very low at day 3, but higher at day 4 and 5. At day 4 there is a higher amount of hydroxyproline in the zinc supplemented group than in the control group.

The specific activity of hydroxyproline in the sponges is lower in the zinc supplemented animals than in controls at day 4 and 5.

Cell composition of early granulation tissue (IV)

At day one the majority of cells in the sponge is polymorphonuclear (PMN) leucocytes. Their relative number decreases gradually during the observation time. Mononuclear cells appear on day 2 and by day 3 fibroblasts and capillaries can be identified.

Histochemically alkaline phosphatase appears almost exclusively located in the PMN leucocytes throughout the observation period.

The activity of alkaline phosphatase in sponge homogenates is high on day 1 and decreases during the observation time both in control and zinc supplemented animals. By day 4 the activity is lower in animals given zinc.

The decreasing enzyme activity is in accordance with the histologically observed gradual disappearance of PMN leucocytes in the developing granulation tissue.

Histochemically acid phosphatase and β -glucuronidase are found mainly in the mononuclear cells especially the macrophages.

Acid phosphatase activity increases during the observation time. No difference is seen between control and zinc supplemented animals.

The activity of β -glucuronidase is low on days 1, 2 and 3. Increased activity is found by day 4. No difference is seen between control and zinc supplemented animals.

DISCUSSION

Chronic zinc deficiency impairs growth, sexual maturity and reparative processes (Prasad, 1963b; Hallböök & Lanner, 1972; Sandstead & Shepard, 1968). In chronically zinc deficient patients and animals zinc supplementation improves or restores growth and healing to normal (Sandstead et al., 1967; Hallböök & Lanner, 1972; Pallauf & Kirchgessner, 1971). Zinc supplementation to patients and animals with normal serum zinc does not influence healing (Hallböök & Lanner, 1972; Sandstead et al., 1970).

During the last decade it has been reported that trauma and infection cause an acute decrease in serum zinc concentration (Sef-ton et al., 1974; Hallmans, 1978; Pekarek & Beisel, 1969).

The functional importance of acute decrease in serum zinc is not known. There is, however, some indications that reparative processes may be impaired by such lowering of serum zinc (Larson et al., 1970).

The present investigation (I) has confirmed earlier observations that trauma is accompanied by a decrease in serum zinc concentration related to the magnitude of the injury. The lowest zinc concentration occurs already 6 hours after major trauma and zinc values then return to normal within 3-4 days.

The mechanism behind the acute decrease in serum zinc concentration is not fully clarified. Urinary zinc excretion has been shown to increase after surgical trauma (Fodor et al., 1973; Sef-ton et al., 1974; Hallböök & Hedelin, 1977) but the amounts lost in the urine postoperatively can hardly explain the decrease in serum zinc. Further, the facts that serum zinc reaches its lowest value already 6 hours after operation and that the return towards

normal occurs while urinary excretion of zinc is still high, indicates that the postoperative decrease in serum zinc cannot be completely explained by urinary loss of zinc.

It has been demonstrated that liver zinc increases after trauma and infection (Pekarek et al., 1972; Hallmans & Wing, 1979). Redistribution of zinc within the body with accumulation in the liver thus might explain part of the acute decrease in serum zinc concentration. It has been speculated that increase in liver zinc is of importance for the increased synthesis of acute phase reactants (C-reactive protein, α_1 -antitrypsin etc.) in the liver after trauma (Pekarek et al., 1972). Accumulation of zinc in the wound area (Savlov et al., 1962) and loss of zinc in wound exsudate (Larson et al., 1970) have also been discussed as causes for the decrease in serum zinc concentration after trauma.

The present investigation does not allow conclusions as to which of these mechanisms that is wholly or mainly responsible for the post-traumatic decrease in serum zinc concentration, but it is possible that all of these mechanisms do contribute.

Preoperative per oral zinc supplementation to patients with normal serum zinc values (II) for 4 weeks results in supernormal serum zinc concentrations. It is thus possible to increase the serum zinc concentration by increase of the dietary content of zinc. This confirms data published by Abdullah et al. (1973) but is contradictory to the observations made by Hallböök & Lanner (1972) who studied patients with chronic leg ulcers. Hallmans & Wing (1979) found that absorption of zinc from the gastrointestinal tract decreases when zinc is administered by other routes. This may explain why oral supplementation of zinc must be maintained

for several weeks before increase of serum zinc can be registered.

The postoperative decrease in serum zinc is of the same order of magnitude in patients with preoperative zinc supplementation as in non-supplemented patients. Due to the high preoperative serum zinc values, however, the lowest values 6 hours after operation remain within the normal range for serum zinc. It seems possible to avoid subnormal serum zinc concentration postoperatively by preoperative administration of zinc.

A detailed analysis of the influence of supplementation with zinc in connection with trauma on reparative processes requires experimental studies.

Rats were chosen as experimental animals because they are commonly used in wound healing studies, are convenient to work with and rather large groups can be used since they are relatively inexpensive. Further, biological variation can be diminished by using rats from one specific strain.

Wound healing was studied by analysis of newly formed granulation tissue. Granulation tissue can be obtained by subcutaneous implantation of sponge material (Boucek & Noble, 1955; Viljanto & Kulonen, 1962) or cylinders (Schilling et al., 1959; Hunt & Zederfeldt, 1967). Viscose cellulose sponges and polyvinyl sponges have been used in different studies. Granulation tissue obtained in the different kinds of sponge material cannot be compared directly. Viscose cellulose sponge causes more pronounced inflammatory reaction and fibroblast proliferation than does polyvinyl sponge (Tengrup, unpublished observations). The size and shape of the sponge and the pore size of the sponge influence the results (Viljanto, 1964; Pallin et al., 1975; Salvatore et al., 1962).

In the present investigation polyvinyl material was used as this material contains very little sponge bound zinc (0-1 $\mu\text{g}/$ sponge) and as zinc cannot be liberated from the sponge by EDTA or strong acid.

It has been shown by Viljanto (1964) that granulation tissue formed within sponges corresponds to the granulation tissue formed in healing wounds. One advantage with sponge implantation technique is that newly formed granulation tissue can be studied without contamination of pre-existent tissue. This is of special importance when studying collagen concentration and metabolism as is done in the major part of the present investigation. The sponge implantation technique also offers possibilities for studies of the early phases of healing which is otherwise difficult.

The operative trauma consists of implantation of 6 sponges subcutaneously in each rat. The influence of this operative trauma on serum zinc concentration could not be studied during the first postoperative day since serial sampling of blood for serum zinc determination cannot be made in these small animals. It has been shown that a comparable trauma, such as creation of open wounds on the back of rats, causes a decrease of serum zinc concentration postoperatively (Hallmans & Wing, 1979). In another study (Tengrup, to be published) it has been shown that the trauma of sponge implantation causes increase of acute phase reactants postoperatively.

Granulation tissue formation and composition was compared in animals with and without zinc supplementation. Zinc supplementation was given by intramuscular injection in order to avoid differences in intestinal absorption. The daily amount of zinc given

intramuscularly is about the same as the calculated daily gastrointestinal uptake of zinc under normal conditions.

In the development of granulation tissue three phases can be identified: the inflammatory phase with duration of 3-4 days, the fibroplasia phase lasting to about day 14 and followed by the maturation phase.

During the inflammatory phase wound fluid with inflammatory cells, initially polymorphonuclear granulocytes and later mononuclear cells accumulates in the sponges. Fibroblasts begin to appear about the third day and at the same time capillaries grow into the sponge pores. The fibroblasts produce collagen which is deposited in the intercellular matrix. Collagen accumulation occurs during the fibroplasia phase. During the maturation phase the amount of collagen does not increase further.

Mechanical strength of granulation tissue is closely related to the amount and the structure of collagen. During the fibroplasia phase strength increase is parallel to the accumulation of collagen (Sandberg & Zederfeldt, 1963). Strength increases further during the maturation phase despite constant amount of collagen. This increase of strength is due to rebuilding of the collagen network in the granulation tissue.

The amount of collagen in a sponge at a given time is the net result of the synthesis of collagen by fibroblasts and degradation of collagen (collagenolysis). The amount of collagen in the sponges can be determined as the amount of hydroxyproline, which is an amino-acid almost exclusively found in collagen (Neuman & Logan, 1950). Hydroxyproline forms a constant part of the collagen molecule and determination of hydroxyproline thus enables calculation

of the amount of collagen present.

The collagen content was determined in ivalon sponges implanted from 4 to 42 days. The reason for studying this period was that earlier studies (e.g. Sandstead & Shepard, 1968; Rahmat et al., 1974) have demonstrated that zinc deficiency in rats resulted in reduced tensile strength of wounds during this period of healing.

Zinc supplementation to normal rats, starting preoperatively and continuing through the experimental period, resulted in higher amounts of hydroxyproline in sponges implanted for 4 days. At later intervals no effect of zinc supplementation was observed. These findings indicate that the effect of zinc supplementation is on the early events of granulation tissue formation. No effect on collagen accumulation in implanted sponges was observed when zinc supplementation was given merely postoperatively. This finding further supports that zinc influences the early events of healing.

In the postoperative course the availability of zinc might thus be of importance either for the inflammatory phase or for the early part of the fibroplasia phase.

As indicated above the net amount of collagen in the sponge at a certain time is the net result of collagen synthesis and collagen degradation. The finding of increased collagen amounts after 4 days of implantation motivates study of the collagen synthesis during the early phase of fibroplasia. Collagen synthesis was studied by *in vivo* pulse labelling technique. ^{14}C -proline was injected intravenously and 24 hours later the amount of radioactive hydroxyproline in the granulation tissue within the sponges was determined. The hydroxyproline in collagen derives from hydroxylation of a certain percentage of proline residues incorporated in

peptide linkages of the procollagen molecules (Prockop et al., 1979). Hydroxyproline as such is never incorporated into collagen (Stetten, 1949). These conditions make it possible to study the synthesis of collagen by determination of radioactive hydroxyproline in collagen with labelled proline as precursor. The amount of radioactive hydroxyproline thus reflects the amount of collagen produced in the granulation tissue during the 24 hour period. Previous studies (e.g. Jiborn, 1980) have shown that 24 hours' *in vivo* incorporation time is suitable for this type of study.

The use of pulse labelling technique *in vivo* can give erroneous results of the rate of collagen synthesis if marked changes occur in the size of the amino-acid pool. Therefore a control study of collagen synthesis in granulation tissue was performed *in vitro* where amino-acid pools are constant.

The study of collagen synthesis (V) shows no effect of zinc supplementation on the rate of collagen or protein synthesis. Increased amounts of collagen after 4 days of implantation (IV, V) despite the same rate of synthesis of collagen indicate that lysis of collagen is reduced in the early phase of healing when zinc supplementation is given.

The specific activity of collagen is a measure of labelled collagen in relation to total collagen which include both labelled and not labelled collagen formed before injection of the radioactive proline.

It was found (V) that the specific activity of collagen was lower after zinc supplementation. This means that sponges from zinc supplemented animals contain more collagen formed before labelling. Since the rate of synthesis of collagen was not altered

this must mean a decreased degradation of the early formed collagen in animals given zinc supplementation.

Previously degradation of collagen has been shown to occur in older granulation tissue where also collagenase activity has been demonstrated (Riley & Peacock, 1967). The present study offers evidence that degradation of collagen occurs concomitant with collagen synthesis also in the early phase of granulation tissue formation.

It has been reported that procollagen might be degraded intracellularly (Bienkowski et al., 1978). Intracellular degradation of procollagen leads to liberation of hydroxyproline. Differences in intracellular procollagen degradation cannot explain the differences in specific activity observed in this investigation as free hydroxyproline is removed during the preparation of the samples. Therefore, the observations made in paper V must mean that there are differences in extracellular degradation of collagen after zinc supplementation.

Mature collagen can be degraded only by collagenase (Harris & Krane, 1974a) which results in a cleavage of the collagen triple helix into smaller fragments (Eisen et al., 1968; Harris & Krane, 1974b). These fragments can be further degraded by unspecific collagenolytic enzymes. Newly formed collagen may be attacked by other enzymes than specific collagenase, e.g. elastase and cathepsins.

Collagenolytic enzymes have been demonstrated in fibroblasts (Werb & Burleigh, 1974), bacteria (Gallop et al., 1957) and in polymorphonuclear leucocytes (Lazarus et al., 1972; Ohlsson & Olsson, 1973). As polymorphonuclear leucocytes are numerous in

early granulation tissue they may be the main source of collagenolytic enzymes.

The finding of decreased collagen degradation in zinc supplemented animals motivates study of the cell composition and enzyme activities in early granulation tissue with and without zinc supplementation. Early granulation tissue was studied using histological, histochemical and biochemical techniques. The histochemical techniques were used only for localization of intracellular enzymes. Quantification of enzyme activity was wholly based on biochemical determinations.

Activities of enzymes were studied in sponge homogenates (IV). Biochemical activity of alkaline phosphatase decreased more rapidly in zinc supplemented animals. Histochemically the activity of alkaline phosphatase was localized to the polymorphonuclear granulocytes. Thus, the lower activity of alkaline phosphatase after zinc supplementation indicates a quicker disappearance of polymorphonuclear granulocytes from the granulation tissue. Histological preparations gave some support for this opinion but allowed no quantification of the number of polymorphonuclear leucocytes present.

The mononuclear inflammatory cells and the fibroblasts were rather few. The activities of acid phosphatase and β -glucuronidase localized mainly to these cells were also low. Thus, no definite conclusions could be drawn about the effect of zinc supplementation on these cells.

The lesser degradation of collagen observed in granulation tissue from zinc supplemented animals may well be related to the changes in polymorphonuclear cell population.

Since polymorphonuclear cells are of importance for the defense against invading bacteria the more rapid disappearance of these cells might mean that zinc supplementation does impair the defense against infection. This problem will be the subject of further studies.

CONCLUSIONS

The present investigation has shown that:

- serum zinc decreases in connection with major surgical trauma,
- preoperative peroral zinc supplementation in man results in supernormal serum zinc values,
- postoperative decrease in serum zinc is of the same magnitude in patients given preoperative zinc supplementation but due to supernormal preoperative serum zinc concentration the postoperative values still are within normal range,
- zinc supplementation started preoperatively and continued through the period of study results in higher amounts of collagen in early granulation tissue but has no effect on older granulation tissue,
- zinc supplementation given merely postoperatively has no effect on the amounts of collagen in granulation tissue,
- zinc supplementation does not influence the rate of collagen synthesis in granulation tissue,
- zinc supplementation decreases collagen degradation in early granulation tissue,

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I

Changes in serum Zinc during and after Surgical procedures

I. Tengrup and H. Samuelsson

CHANGES IN SERUM ZINC DURING AND AFTER SURGICAL PROCEDURES

Ingrid Tengrup and Håkan Samuelsson

From the Department of Surgery, Malmö General Hospital, University of Lund, Malmö, Sweden

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Abstract. Serum zinc was followed in 49 patients during and after minor and major surgery. Serum zinc decreases significantly after major operative trauma such as SPV and cholecystectomy whereas no change can be seen after minor surgery such as hernia repair. The drop is most pronounced 6 hours after the operation. Serum zinc gradually returns to normal level in 2–3 days. In older patients the decrease is more pronounced but return to normal serum zinc level occurs as quickly as in young patients.

The significance of zinc for normal growth, sexual maturity and preservation of intact skin and hair has long been known in veterinary medicine (Tucker & Salmon, 1955; Miller, 1970). It is, however, only during the last decade that there has been an interest for zinc in human medicine. In several papers (e.g. Husain, 1969; Greaves & Skillen, 1972; Hallböök & Lanner, 1972) it has been demonstrated that peroral zinc treatment increases the rate of healing of chronic leg ulcers in patients with low serum zinc levels. No such positive effect was found in patients with normal levels of serum zinc. It often takes several weeks before the positive effect of zinc therapy can be demonstrated (Haeger et al., 1969). There are also papers indicating that the healing of non-chronic wounds may be stimulated by extra supply of zinc. Thus, Pories et al. (1967) found an increased rate of healing in young, healthy men operated on with excision of inflamed pilonidal sinus when extra zinc was given orally, as compared with an untreated control group. Further, Larson et al. (1970) have demonstrated improved healing in patients with burns after extra zinc supply.

Experimental studies have shown that extra quantities of zinc influence the inflammatory reaction (Chvapil, 1973; Chvapil et al., 1973) and stimulate early collagen formation in granulation tissue

(Tengrup & Zederfeldt, 1975). Thus, not only a chronic zinc deficiency but also a transitory reduction of serum zinc level might be of importance for the rate of healing. Decrease of serum zinc levels after burns has been demonstrated by Larson et al. (1970). Fodor et al. (1973) showed a minor drop in serum zinc concentration during short-time anesthesia, but a marked drop in patients subjected to hysterectomy during full anesthesia. The drop was demonstrated already 15 min after the start of the operation and a low level remained during the whole operation. They also reported an increased excretion of zinc in urine, occurring already during operation and persisting for the first postoperative days. Reports of decreased serum zinc concentration in connection with operative trauma and of increased urinary excretion of zinc postoperatively have also been given by Sefton et al. (1974) and by Hallböök & Hedelin (1975).

The present study reports changes in serum zinc concentration during and after surgical procedures of different magnitude.

MATERIAL AND METHODS

Serum zinc levels were followed in 49 patients. The patients fall into five different groups:

- I. A control group of nine patients subjected to bronchoscopy or oesophagoscopy in full anesthesia.
- II. Seven patients subjected to inguinal hernia repair in full anesthesia.
- III. Eight patients subjected to inguinal hernia repair in spinal anesthesia.
- IV. Thirteen patients operated with selective proximal vagotomy.
- V. Thirteen patients subjected to cholecystectomy for gall bladder disease. Two of these patients also had cholecystectomy due to common-duct stone.

The details about time of anesthesia, time of operation and mean age are given in Table I.

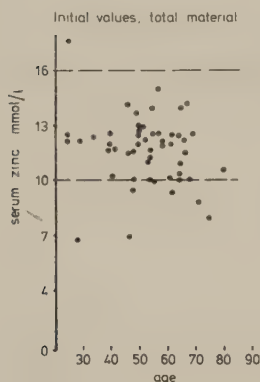


Fig. 1

Premedication was given on an individual basis. All patients in group V were given costal nerve blockade with Marcain®-adrenaline. Fluid was given peroperatively. Blood transfusion was given to one patient in group IV at the end of the operation.

Blood samples for determination of serum zinc concentration were taken on admittance to the hospital, after induction of anesthesia just before surgery was started, after 30 min of surgery and/or immediately postoperatively. Further, samples were taken six hours after the conclusion of the operation, in the morning of the following postoperative days and at the first ambulatory control after the operation. In the control group, blood samples were also taken three hours postoperatively. All samples from one patient were saved and analysed at the same occasion. Serum albumin concentration was determined preoperatively and on the first and third or fourth postoperative day.

The blood samples have been taken with disposable needles into acid-washed glass tubes provided with acid-washed plastic stoppers. Only samples free from hemolysis were examined. Serum zinc concentration has been

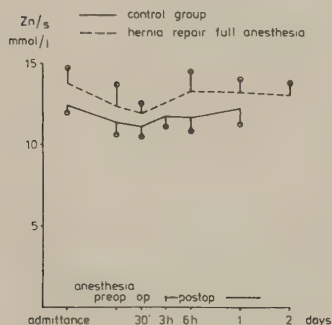


Fig. 2

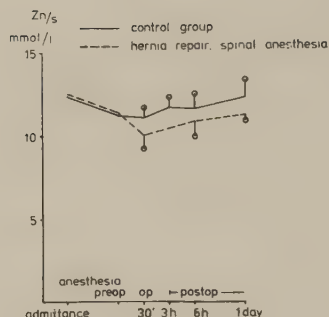


Fig. 3

determined with atomic absorption technique according to the method described by Parker et al. (1967). Normal value at our laboratory is 13 mmol/l with a range of 10–16 mmol/l.

RESULTS

The serum zinc levels on admittance to the hospital are given in Fig. 1. In six patients abnormally low values were found. However, mean values for the different groups were all within the normal range. The initial values were independent of the age of the patient. The serum zinc levels just before surgery was started were about 10% below the initial level in all groups.

The control group showed no changes in serum zinc levels during and after operation. The two hernia groups had values corresponding to the control group (Figs. 2 and 3). Between the two hernia groups there is, however, a slight difference. After 30 min of operation, and 6 hours postoperatively,

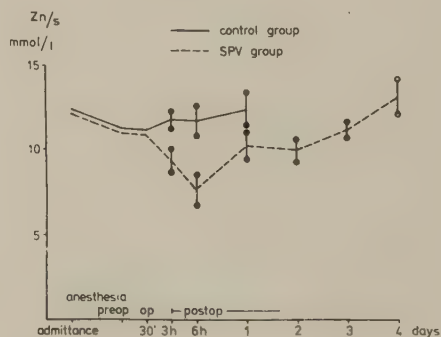


Fig. 4

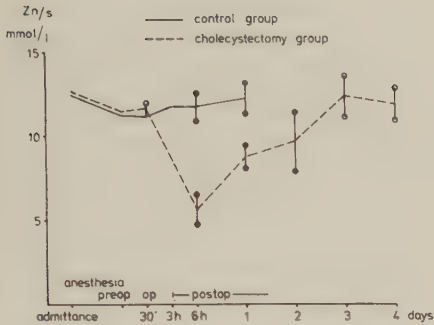


Fig. 5

the spinal anesthesia group has somewhat lower serum zinc concentration than the other hernia group.

In groups IV and V there was a marked decrease in serum zinc, most pronounced in the cholecystectomy group (Figs. 4 and 5). The lowest mean value was found six hours postoperatively. Thereafter, serum zinc increased gradually and returned to normal level on the 3rd or 4th postoperative day. Serum zinc in one patient with an admittance value of 13.2 mmol/l, fell to 1.5 mmol/l six hours postoperatively and was only 8.7 mmol/l on the 4th postoperative day. The decrease in serum albumin concentration was in this patient 28%, more than the average decrease. Another patient in group V had a low initial serum zinc of 8.7 mmol/l and a serum albumin of 47 g/l. The changes in serum zinc during and after operation were, in this patient, quite similar to the pattern in the other patients, but

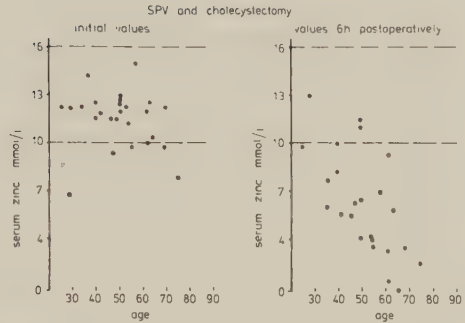


Fig. 6

on a lower level. On the 8th postoperative day the serum zinc in this patient was 9.8 mmol/l.

The older patients seem to have a more pronounced decrease six hours postoperatively than the younger ones (Fig. 6). However, return to normal values was as rapid in the old patients as in the young patients.

One patient in group IV developed a wound infection on the sixth postoperative day, but this did not affect the serum zinc level. All other patients healed without complications.

DISCUSSION

No difference in the initial serum zinc values relating to age has been found in this study. This is in contrast to what Hallböök & Hedelin (1975) have reported. They found lower serum zinc concentrations in patients over 60 years of age than in

Table I

Group	I	II	III	IV	V
Operation type	Endoscopy (+px)	Hernia repair		SPV	Cholecystectomy
Mean operation time, min	20	60	55	90	70
Anesthesia type	Full anesthesia	Spinal anesthesia	Full anesthesia without intubation		Full anesthesia
Anesthesia drugs	Intubation Pentotal, N ₂ O Fentanyl Suxameth chlor	Tetracain	Pentotal, N ₂ O Halothane		Intubation Pentotal, N ₂ O Lealgin or Fentanyl, Suxameth.chlor and/or Pavulon
Mean anesthesia time, min	30	—	80	130	100
Age	40–70 years	50–80 years	19–56 years	40–65 years	25–75 years
Number of patients	9	8	7	13	13

younger patients. This study, on the other hand, showed a more marked reduction of serum zinc after major trauma in older than in younger patients.

The zinc values just before starting surgery are in all groups about 10% below the initial values. This decrease corresponds well with the changes in serum zinc reported by Fodor et al. (1973) during short anesthesia. The decrease in serum zinc can be explained by the concomitant decrease of serum albumin resulting from confinement to bed. The small initial decrease in serum zinc level seems, thus, not to be related to anesthesia, as was claimed by Fodor et al. (1973).

No difference in serum zinc concentration was found between the control group and either of the hernia repair groups. This again indicates that anesthesia does not influence serum zinc levels.

The hernia repair groups representing minor operative trauma do not differ in zinc values from the control group with no operative trauma.

In the groups representing major operative trauma there are marked differences from the control group, indicating that the degree of trauma is of importance for the postoperative decrease in serum zinc level. These findings correspond well with the findings of Sefton et al. (1974), who reported low serum zinc concentrations after major trauma.

It is obvious, therefore, that serum zinc decreases after major trauma, but where, then, does it go? In this investigation no registration of zinc excretion in urine has been done. Hallböök & Hedelin (1975) found a doubling of urine zinc excretion during the first postoperative days, and Fodor et al. (1973) reported an increase to between six and eight times the normal values in urine zinc during the same period. Since Fodor and co-workers have not reported the method for urine sampling, it cannot be excluded that these very high values depend on some contamination, because considerable technical problems are involved in collecting urine from 24 hours in acid-washed containers during the first postoperative days, especially in women. In any case the increased amount of zinc in urine postoperatively does not correspond to the amount of zinc disappearing from serum. Moreover, return of serum zinc levels towards normal starts while urine excretion is still high and when no exogen supply of zinc has been given. Therefore, a redistribution of available zinc in the organism in answer to major trauma seems reasonable.

Pekarek & Beisel (1969) and Pekarek et al. (1972) claim that following trauma or infection there is a disappearance of zinc from serum and an increased uptake of zinc by the liver and the RES. The increased zinc level in the liver could be of importance for the increased protein synthesis, especially of acute phase reactants such as fibrinogen, orosomucoid and haptoglobin. From animal experiments it is also known that zinc is of importance for many enzyme systems involved in DNA, RNA and protein synthesis (Sandstead & Rinaldi, 1969; Williams & Chesters, 1970; Prasad et al. 1971).

Another possibility is that zinc accumulates in the operative field. It is at present not known if this is the case.

Savlov et al. (1962), using radioactive zinc, found high activity in a wound region compared to corresponding untraumatized regions. However, this can be due to the increased vascularity in the traumatized area and does not necessarily mean that zinc available for the healing process is increased. It may well be that the pronounced drop in serum zinc during and after trauma results in too little zinc available locally in the traumatized region. If so, healing may be delayed as a consequence of impaired inflammatory reaction, since Chvapil (1973) showed that zinc deficiency may reduce the phagocytotic activity of macrophages. An explanation for the reported increased rate of healing and the earlier start of collagen synthesis after extra supply of zinc preoperatively could then be that this treatment renders a more optimal concentration of zinc in the wound. If so, it would be of interest to study whether an extra supply of zinc before surgery could increase the rate of healing, especially in elderly patients where the drop in serum zinc after operation is most pronounced.

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II

Serum Zinc before and after cholecystectomy in Zinc-Treated patients

I. Tengrup and B. Zederfeldt

SERUM ZINC BEFORE AND AFTER CHOLECYSTECTOMY IN ZINC-TREATED PATIENTS

Ingrid Tengrup and Bengt Zederfeldt

*From the Department of Surgery, Malmö General Hospital,
University of Lund, Malmö, Sweden*

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Abstract. Nine female patients were given zinc sulphate (Solvezinc®) 220 mg three times daily for 4 weeks prior to cholecystectomy. Mean serum zinc before zinc treatment was $17 \pm 0.8 \mu\text{mol/l}$ and rose to $25 \pm 1.6 \mu\text{mol/l}$ after zinc treatment. 6 hours postoperatively serum zinc was $13 \pm 1.4 \mu\text{mol/l}$. Thereafter it gradually increased to $20 \pm 0.7 \mu\text{mol/l}$ by the third postoperative day. The present investigation thus shows that pretreatment with zinc increases the serum zinc level but does not prevent the drop in serum zinc in connection with cholecystectomy. However, with the pretreatment serum zinc never falls below the normal range.

Depressed serum zinc levels have been reported in connection with anesthesia and operative trauma (Fodor et al., 1973; Sefton et al., 1974). In a previous study (Tengrup & Samuelsson, 1977) we were able to demonstrate a postoperative drop in serum zinc related to the magnitude of the operative trauma. During and after anesthesia alone and after hernia repair in general anesthesia no change in serum zinc was observed. After cholecystectomy there was, however, an early marked drop in serum zinc with the lowest value 6 hours postoperatively. Serum zinc then gradually increased and returned to normal on the third postoperative day.

The present study was performed to determine whether peroral pretreatment with zinc can abolish the postoperative drop in serum zinc after cholecystectomy.

MATERIAL AND METHODS

Nine female patients (mean age 46 years; range 35–58 years) for whom cholecystectomy was planned were administered zinc sulphate (Solvezinc®) 220 mg three times daily for 4 weeks prior to operation. One patient stopped taking the preparation because of gastrointestinal disturbances; the other 8 patients continued taking the preparation for the whole period without any side effects.

Seven patients underwent simple cholecystectomy. In one patient a common duct stone was removed. Mean

anesthesia time was 140 (range 90–170) minutes and mean operation time was 100 (range 60–140) minutes. The operations were performed under general anesthesia and all patients had costal nerve blockade preoperatively.

Serum zinc concentrations were determined before and after 4 weeks of zinc treatment. In addition, samples were taken immediately after conclusion of the surgical procedure, 6 hours later, and in the mornings on the 3 subsequent postoperative days.

Serum albumin was determined on admission to hospital and on the first postoperative day.

The blood samples were drawn with disposable needles into acid-washed glass tubes provided with acid-washed plastic stoppers. Serum zinc concentration was determined with atomic absorption technique using the method described by Parker et al. (1967). Normal range of serum zinc at our laboratory is $10\text{--}16 \mu\text{mol/l}$, mean value $13 \mu\text{mol/l}$.

RESULTS

Mean serum zinc concentration before zinc treatment was $17 \pm 0.8 \mu\text{mol/l}$ (Fig. 1), rising after 4 weeks of zinc treatment to $25 \pm 1.6 \mu\text{mol/l}$. Serum albumin at this time was $48 \pm 0.6 \text{ g/l}$.

At the conclusion of the operation mean serum zinc concentration was 20 ± 0.6 and 6 hours later $13 \pm 1.4 \mu\text{mol/l}$. Thereafter it gradually increased to $20 \pm 0.7 \mu\text{mol/l}$ by the third postoperative day. Serum albumin was $47 \pm 1.3 \text{ g/l}$ on the first postoperative day. Mean blood loss during operation was $0.2 \pm 0.03 \text{ l}$ and drainage loss during the first postoperative day was $0.05 \pm 0.01 \text{ l}$. The values are given as mean $\pm$ standard error of the mean.

The postoperative course was uneventful in all patients and all healed without complications.

DISCUSSION

Previous investigations have shown that it is possible to normalize a low level of serum zinc by peroral

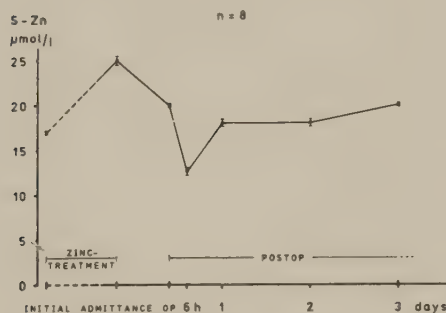


Fig. 1. Serum zinc before, during and after cholecystectomy in zinc-treated patients. Oral zinc raises serum zinc levels but does not prevent postoperative decline.

zinc treatment (Greaves & Skillem, 1970; Hallböök & Lanner, 1972). On the other hand, there has been uncertainty whether it is possible to raise a normal serum zinc by peroral zinc treatment. Hallböök (1974) found no increase in serum zinc after 18 weeks of zinc medication in patients with initially normal serum zinc. Abdullah et al. (1973) showed that increase in serum zinc levels was slight during the first 4 weeks of oral zinc treatment but after 6 weeks serum zinc had increased from 100 to 150 µg/100 ml. The activity of δ -amino-levalulinic acid dehydratase in human red blood cells, an enzyme activated by zinc, did not increase until after 4 weeks of zinc treatment. The difference in findings by Hallböök and Abdullah might be a question of how consistently the patients have taken the zinc preparation.

The present study shows that it is possible to raise the serum zinc level with 4 weeks of oral zinc treatment.

Mean serum zinc before zinc treatment was slightly above the normal range. The samples are taken 12 hours after the last meal. This may explain the high values since it is known that food intake results in a 5–10% decrease of serum zinc (Davies et al., 1968).

The present investigation further shows that pre-treatment with zinc does not prevent a drop in serum zinc in connection with major operative trauma (Fig. 2). However, although the drop occurs in the patients pretreated with zinc, serum zinc never falls below normal range of serum zinc. Serum zinc concentration returns spontaneously to

the level it had before zinc treatment without any extra supply of zinc. There is no change in serum albumin on the first postoperative day.

Fodor et al. (1973) and Hallböök (1977) reported an increase in urine zinc during the first days after operative trauma. The amount of zinc lost in the urine does not correspond to the amount of zinc disappearing from serum. Moreover, serum zinc increases again while urine zinc is still high. The decrease in serum zinc can thus not be explained solely by urine losses but a redistribution of zinc in the body must occur.

Pekarek et al. (1972) showed an increased uptake of zinc by the liver after infection and trauma. Experimental studies by Hallmans (1978) show that liver zinc increased in rats with excisional wounds concomitant with a decrease in serum zinc. Hallmans & Wing (1978) also showed that there is no difference in the zinc content of other organs between non-operated and operated rats. Thus there is evidence of a specific increase in zinc in the liver in response to trauma and stress. It has been suggested by Beisel et al. (1976) that the increased liver zinc levels after stress are of importance for the increased protein synthesis there, especially of so-called acute phase proteins. It is known from animal experiments that zinc is of importance for enzyme systems involved in the protein synthesis (William & Chesters, 1970). The spontaneous return of serum zinc after trauma to normal level might be explained by an increased level in serum of newly synthesized α_2 -macroglobulin liberated from the liver. α_2 -macroglobulin is a zinc-containing

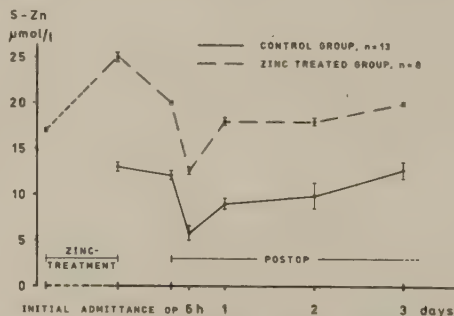


Fig. 2. Serum zinc changes after cholecystectomy. Control material from Tengrup & Samuelsson, Acta Chir Scand 143, 195, 1977. Postoperative serum zinc levels in zinc-treated patients remain within normal range.

protein which binds zinc strongly and under normal conditions accounts for one-third of serum zinc (Parisi & Vallee, 1970).

In this investigation no registration of liver zinc content was made. Since pretreatment with zinc increases serum zinc it seems reasonable to assume that liver zinc is also increased. Richards & Cousins (1976) have shown experimentally in rats that liver zinc increases after administration of zinc either perorally or parenterally. Presumed increased availability of zinc in the liver of patients in this investigation does obviously not abolish the redistribution of zinc after trauma.

During recent decades it has been demonstrated that wound healing is delayed in patients with low serum zinc. Normal healing can be restored by extra supply of zinc perorally to patients with long-term low serum zinc (Haeger et al., 1969; Hallböök & Lanner, 1972). It is not known if acute decrease in serum zinc in connection with trauma influences wound healing. Larson et al. (1970) found that serum zinc in burned children was low and that oral zinc supplementation improved the rate of healing of burned skin and improved the take of skin grafts. Therefore it might be beneficial to supplement with zinc in connection with trauma.

Zinc treatment decreases the amount of granulocytes in wounds (Hallmans, 1978) and inhibits mobilization of both polymorphonuclear cells and macrophages (Chvapil, 1976; Chvapil et al., 1977).

These findings indicate that zinc might be of importance for the inflammatory phase of wound healing, and in that case even a transient early decrease in serum zinc postoperatively could be of importance. It is hardly possible in clinical investigations to study further the importance of zinc for the early wound healing and therefore experimental studies in rats have been initiated.

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III

Granulation tissue formation in Zinc-Treated rats

I. Tengrup, J. Ahonen and B. Zederfeldt

GRANULATION TISSUE FORMATION IN ZINC-TREATED RATS

Ingrid Tengrup, Juhani Ahonen and Bengt Zederfeldt

*From the Department of Surgery, Malmö General Hospital,
University of Lund, Sweden*

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Abstract. Chronic zinc deficiency impairs wound healing. It is not known if acute decrease of serum zinc occurring after trauma has the same negative effect. We have studied the effect of extra zinc supply in connection with trauma on granulation tissue formation in subcutaneously implanted polyvinyl sponges. Zinc given postoperatively had no effect on granulation tissue formation. Zinc administered also preoperatively resulted in higher amount of collagen by the fourth postoperative day but not later. This effect of zinc on the collagen content can be explained either by an increased collagen synthesis or by decreased collagen break down. The fact that zinc had no effect when given only postoperatively indicates that collagen synthesis is not affected. The demonstrated effect of zinc thus seems to be due to decreased collagen break down.

Zinc is a necessary cofactor for the functions of enzymes responsible for synthesis of DNA and protein (Williams & Chesters, 1970). It seems reasonable to assume therefore that zinc can be of importance for reparative processes.

A positive effect of zinc supply on wound healing in man was first reported in 1967 by Pories et al. Zinc has since been shown to improve healing of chronic venous leg ulcers (Greaves & Skillen, 1970; Hallböök & Lanner, 1972; Haeger et al., 1974) but only in patients with low serum zinc. In patients with normal serum zinc levels no effect of extra zinc has been shown.

Zinc deficiency in experimental animals causes delayed epithelialization and delayed strength development in wounds. Zinc deficiency also results in lower amounts of collagen in granulation tissue and proportionally larger amounts of soluble collagen (Fernandez-Madrid et al., 1971). Supply of zinc normalizes wound healing in these animals. Thus chronic zinc deficiency seems to impair healing.

In connection with trauma there is an acute decrease in serum zinc in both man and animal (Sefton et al., 1974; Hallmans, 1978). After cholecystec-

tomy the serum zinc drop is most pronounced 6 hours after operation and normal values are restored by the third postoperative day (Tengrup & Samuelsson, 1977). It is not known if this acute decrease in serum zinc impairs healing. The findings by Larson et al. (1970), studying burns, indicate that this may be the case.

The aim of this investigation was to study the effect of extra zinc supply on the granulation tissue formation in normal animals.

MATERIAL AND METHODS

One hundred and eight white female Sprague-Dawley rats, weighing 180-200 g (divided into groups of 9-20 rats) were used (Table I). Five rats died during the experiment and 6 were excluded because of infection. Thirty-nine rats were given 0.5 ml of 0.01 % zinc acetate solution intramuscularly daily with the first injection half an hour before the start of the experiment (Group B). Twenty rats were given the same dose of zinc but with the first injection on the morning of the first postoperative day (Group C). The other rats were controls (Group A). All the animals had free access to pellets and water during the experiment.

Six polyvinyl sponges, weighing 55-60 mg, were implanted subcutaneously on the back of each animal according to Viljanto & Kulonen, 1962. The sponges were removed after 4, 7, 14 and 42 days and were analyzed for collagen and hemoglobin content. Serum zinc was determined. Collagen was determined according to Pikkarainen (1968) as the amount of hydroxyproline in the sponges. Hemoglobin was determined as cyanmethemoglobin as recommended by the International Committee for Standardization in Haematology (1965). Serum zinc was determined with atomic absorption technique.

RESULTS

In the control group the amount of hydroxyproline in the sponges increased during the whole observation time (Fig. 1). In group B (preoperative zinc) the amount of collagen was higher than in controls after 4 days ($p < 0.05$) but after 7, 14 and 42 days there

Table I. Number of animals at each interval

	Days			
	4	7	14	42
Group A: Controls	10 (1†)	9	10	20
Group B: Zinc-treated preop.	10 (3†)	9	10	10
Group C: Zinc-treated postop.	10 (1†)	10		

were no differences against controls. There were no differences between group A (controls) and group C (postoperative zinc).

Serum zinc concentration was the same at all intervals and there were no differences between controls and zinc-treated animals (Fig. 2).

In group A the amount of hemoglobin in the sponges decreased from the fourth to the seventh day. It was again higher by the fourteenth day but decreased again by day 42 when the amount was about the same as on the seventh day. After four days the amount of hemoglobin was lower in group B than in controls ($p < 0.05$) whereas after 14 days it was higher in this group ($p < 0.05$, Fig. 3). Group C did not differ from group A at any of the intervals studied.

DISCUSSION

There are many ways to study wound healing. In man you are mostly restricted to study the healing of incisional wound or wounds healing by second intention. These methods can also be used in animals. There are, however, some limitations with these methods. Firstly, healing is usually evaluated

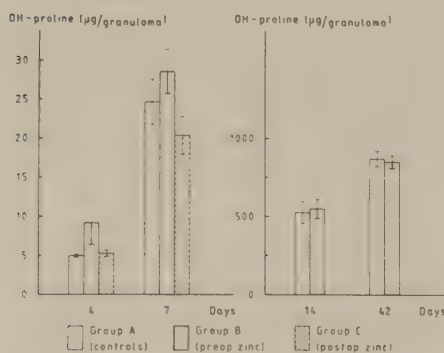


Fig. 1. Hydroxyproline content of the sponges after 4, 7, 14 and 42 days of implantation.

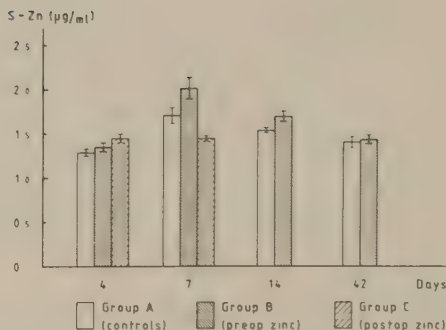


Fig. 2. Serum zinc levels at sacrifice after 4, 7, 14 and 42 days of sponge implantation.

by rather crude parameters such as the time of healing or the tensile strength development. Secondly, they are of limited value during the early phase of wound healing.

A way of studying the formation of granulation tissue more in detail is analysis of pure granulation tissue obtained by implantation of sponges (Woessner & Boucek, 1961; Viljanto & Kulonen, 1962) or cylinders (Schilling et al., 1959) subcutaneously in animals. Different types of sponges can be used. Viljanto & Kulonen (1962) used viscose cellulose sponges whereas Woessner & Boucek (1961) used polyvinyl sponges. The granulation tissue formed in these two different kinds of sponges cannot be compared directly. The viscose cellulose sponge causes a more pronounced inflammatory reaction and fibroblast proliferation than the polyvinyl sponges. The results depend, besides the material of the sponges also on the pore size (Salvatore et al., 1961).

Our results are in accordance with those of Viljanto (1964) and Woessner & Boucek (1961) in as much as the amount of hydroxyproline increased successively during the observation time. The collagen amounts are, however, significantly lower than those found by Viljanto and slightly lower than those found by Woessner & Boucek. The difference against the findings of Viljanto can be explained by the different types of sponges used. Woessner & Boucek who used the same sponge material as we, have few observations at each interval. Our methods for determining collagen also differ and nothing is known about differences in pore size of the sponges.

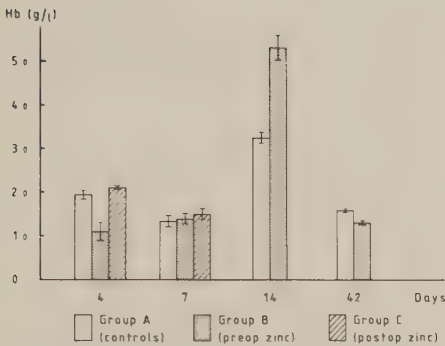


Fig. 3. Hemoglobin content of the sponges after 4, 7, 14 and 42 days of implantation.

As regards the effect of zinc on wound healing, most investigators now agree that chronic zinc deficiency in man as well as in animal impair wound healing and that administration of zinc normalizes healing (Sandstead et al., 1970; Hallböök & Lanner, 1972). In man the effect of zinc is not seen until after 4 weeks of zinc treatment (Haeger et al., 1972).

Administration of zinc to animals and patients with normal serum zinc levels does not improve the rate of healing or the strength development of wounds (Barcia, 1970; Sandstead et al., 1970; Norman et al., 1975). In these investigations the formation of granulation tissue has not been studied biochemically.

Whether the acute decrease in serum zinc concentration in connection with trauma (Tengrup & Samuelsson, 1977) influences the formation of granulation tissue is not known. However, the observations by Larson et al. (1970) that burns did not heal until the serum zinc level had returned to normal indicate that even an acute decrease in serum zinc may be of importance for repair. The decrease in serum zinc reported in connection with trauma (Tengrup & Samuelsson, 1977; Hallmans, 1978) occurs almost immediately. Therefore we chose to start the zinc treatment preoperatively (group B). Zinc was in a separate group (group C) given only after the operation when zinc levels should have passed their lowest point and started to increase again. Zinc was given parenterally to avoid differences in intestinal absorption.

We found that extra supply of zinc parenterally with start preoperatively influences the formation of granulation tissue in the early phase of wound

healing with higher amounts of collagen as a result. No effect was seen in the later phases of wound healing in accordance with other investigators (Sandstead et al., 1970). Zinc treatment starting postoperatively did not influence the granulation tissue formation.

Zinc levels could in these experiments not be determined during the experiment since the method available requires fairly large volumes of serum. The fact that serum zinc levels at sacrifice of animals were not altered thus merely indicates that the amount of zinc given has not lead to abnormally high zinc levels. To which extent early zinc administration influenced the acute changes of serum zinc thus cannot be evaluated.

The increased collagen amount during the early phase of wound healing when extra zinc is given preoperatively can be explained either by an increased collagen synthesis or by a decreased breakdown of collagen. It has been shown by Chvapil et al. (1973 and 1976) that the function of granulocytes and macrophages is influenced by zinc in vitro. These two types of cells are part of the inflammatory reaction that initiates the formation of granulation tissue. The low amount of hemoglobin on the fourth day in group B might indicate a less pronounced inflammatory reaction in this group. The fact that increased collagen amount is found only during the early healing period and the lack of effect of zinc administered postoperatively point to an influence of zinc on the inflammatory reaction. A direct influence on the fibroblasts is contradicted by the fact that no effect of zinc is seen during the fibroplasia phase, e.g. from the fifth to the twentieth day (Dunphy & Jackson, 1962).

This investigation thus shows that zinc administration influences the formation of granulation tissue during the early phase of wound healing. There is strong circumstantial evidence that this is the result of a modification of the inflammatory reaction. Investigations have been initiated to study this problem further by analysis of the effect of zinc on the inflammatory reaction as well as on the collagen synthesis during the early phase of wound healing to evaluate whether the synthesis or breakdown of collagen is affected.

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IV

Cytochemical study of granulation tissue in Zinc-Treated rats

I. Tengrup, J. Ahonen, F. Rank and B. Zederfeldt

ABSTRACT

The effect of zinc administration on cell composition of early granulation tissue has been studied in rats using histological and cytochemical methods. Granulation tissue was obtained by subcutaneous implantation of polyvinyl sponges. The experimental group was given zinc intramuscularly daily beginning preoperatively. Zinc administration does not change the early inflammatory cell infiltration but results in a more rapid disappearance of polymorphonuclear granulocytes from the granulation tissue. No effect can be seen on the mononuclear cell population. The observations are related to alterations in repair processes following zinc administration.

Chronic zinc deficiency impairs wound healing in man (Greaves & Skillen, 1970; Hallböök & Lanner, 1972). In experimental studies it has been shown that zinc deficiency causes delayed epithelialization (Sandstead et al., 1970) as well as delayed strength development in wounds (Sandstead & Shepard, 1968). The delayed strength development can be related to lower amounts of collagen in granulation tissue and proportionally larger amounts of soluble collagen (Fernandez-Madrid et al., 1971).

Previous investigations have shown that serum zinc decreases in connection with trauma (Sefton et al., 1974; Hallböök & Hedelin, 1977; Tengrup & Samuelsson, 1977; Hallmans, 1978). Zinc administration before and after trauma results in higher amounts of collagen in granulation tissue after 4 days, which may indicate that also an acute lowering of serum zinc influences the early formation of

granulation tissue (Tengrup et al., 1979).

The tissue repair process can be divided into three phases: the inflammatory, the proliferative and the remodelling phase, see e.g. Ross (1968). The inflammatory phase passes successively into the proliferative phase with proliferation of fibroblasts and synthesis of collagen.

The effect of zinc on the collagen amount in granulation tissue after 4 days can be explained either by a direct influence on the fibroblasts or by a modification of the inflammatory reaction. Chvapil et al. (1976) have shown that zinc *in vitro* causes changes in migration and phagocytosis of inflammatory cells.

In this investigation we have studied the effect of zinc administration on cell composition of early granulation tissue using histological and cytochemical methods.

MATERIAL AND METHODS

Eighty white female Sprague-Dawley rats, weighing 180-200 g, were used. The rats were divided into groups according to Table I. Two rats died during the experiment and 1 was excluded because of infection (microscopical). All animals had free access to pellets (Astra-Ewos) and water.

To obtain granulation tissue 6 Ivalon sponges, weighing 55-60 mg, were implanted subcutaneously on the backs of the rats according to Viljanlo (1964).

Zinc was given daily intramuscularly as 0.5 ml 0.01% zinc acetate solution with the first injection half an hour before the operation. The control animals received no zinc injections.

TABLE I: Number of animals at each interval.

	Day 1	Day 2	Day 3	Day 4
Group I: Controls	10	10	10	10
Group II: Zinc treated animals	10	10	10	10 (2†, 1 inf)

Sponges were removed 1, 2, 3 and 4 days after implantation and were analyzed for activities of alkaline phosphatase, acid phosphatase and β -glucuronidase (Bessey et al., 1946; Andersch & Szczypinski, 1947; Fishman et al., 1953; Fishman et al., 1948).

The sponges used for microscopical examinations were divided in two halves. One half was prepared by routine histological techniques and stained with hematoxylin-eosin and van Gieson. The other half was rapidly frozen in propane cooled by liquid nitrogen. They were stored at -70° C. Sections about 5 microns thick were processed in a cryostat. The azo-dye technique was used to demonstrate:

Alkaline phosphatase with naphtol AS-TR phosphate as substrate and Fast Red TR as diazonium salt (Aronsen et al., 1968),

acid phosphatase with naphtol AS-TR phosphoric acid (Barka & Anderson, 1963) and

β -glucuronidase with naphtol AS-BI β -D-glucosiduronic acid (Hayashi et al., 1964) as substrates.

Hexazolised fuchsin (pararosanilin) was used as diazonium salt (Barka & Anderson, 1962).

Normal rat liver was used as control for staining technique.

RESULTS

Histologically granulation tissue development is observed at the periphery of the sponges. At day 1 the majority of cells are polymorphonuclear granulocytes. Their relative number decreases gradually during the observation time. Mononuclear cells appear on day 2 and from day 3 fibroblasts and capillaries can be identified.

Alkaline phosphatase

The activity of alkaline phosphatase in sponge homogenates is high on day 1 and decreases during the observation time in both groups. The decrease is more rapid in the zinc treated animals. At day 4 the activity is lower in animals given zinc ($p < 0.05$) (Fig. 1).

Histochemically the activity appears almost exclusively located in the polymorphonuclear leucocytes throughout the observation period. The staining intensity shows a gradual decline most apparent in the zinc treated group.

The decreasing enzyme activity is in accordance with the gradual disappearance of polymorphonuclear leucocytes in the developing granulation tissue.

Acid phosphatase and β -glucuronidase

Acid phosphatase activity increases during the observation time. No difference is seen between control and zinc treated animals (Fig. 2).

The activity of β -glucuronidase is low on days 1, 2 and 3. Increased activity is found at day 4 (Fig. 3). No difference is seen between control and zinc treated animals.

Histochemically acid phosphatase and β -glucuronidase are found

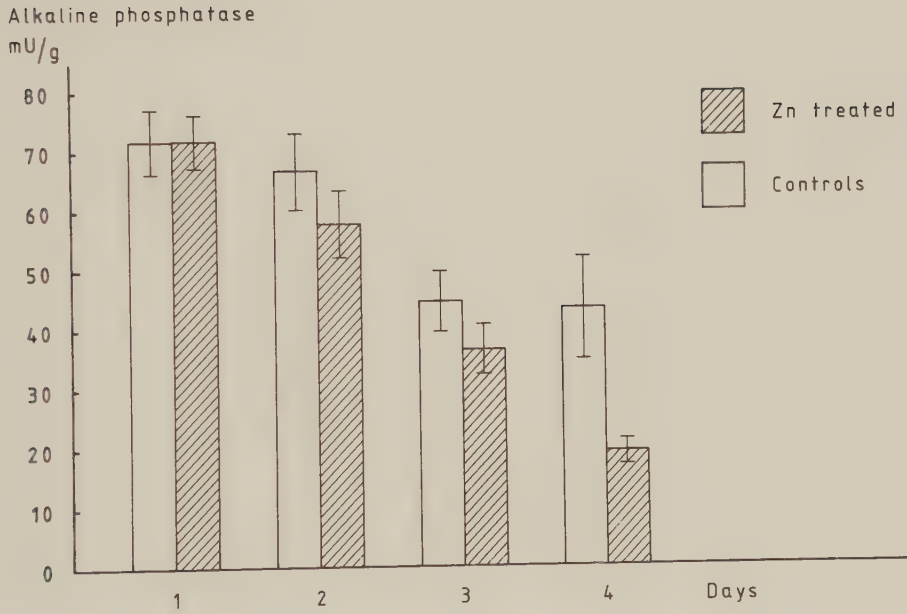


Fig. 1. Activity of alkaline phosphatase in sponge homogenate after 1, 2, 3 and 4 days of implantation.

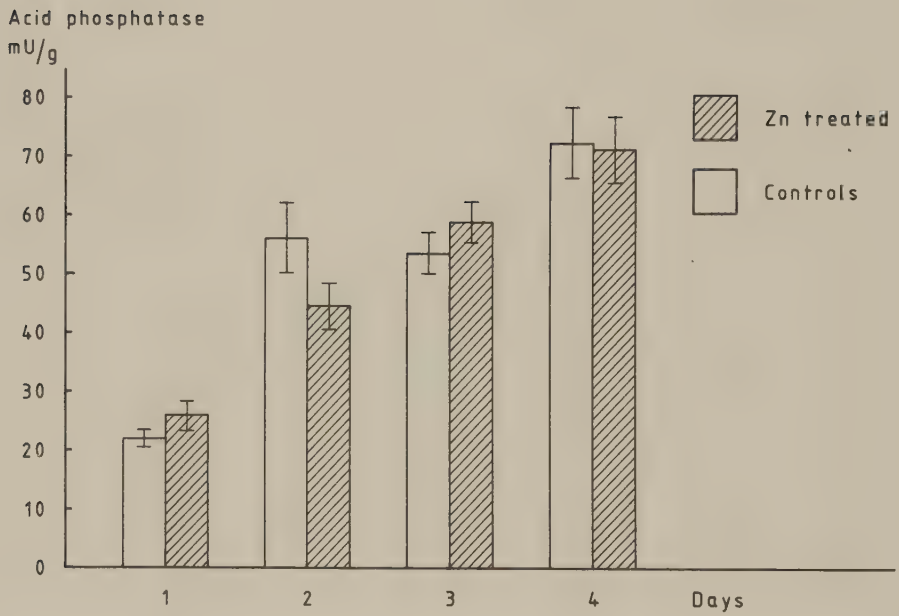


Fig. 2. Activity of acid phosphatase in sponge homogenate after 1, 2, 3 and 4 days of implantation.

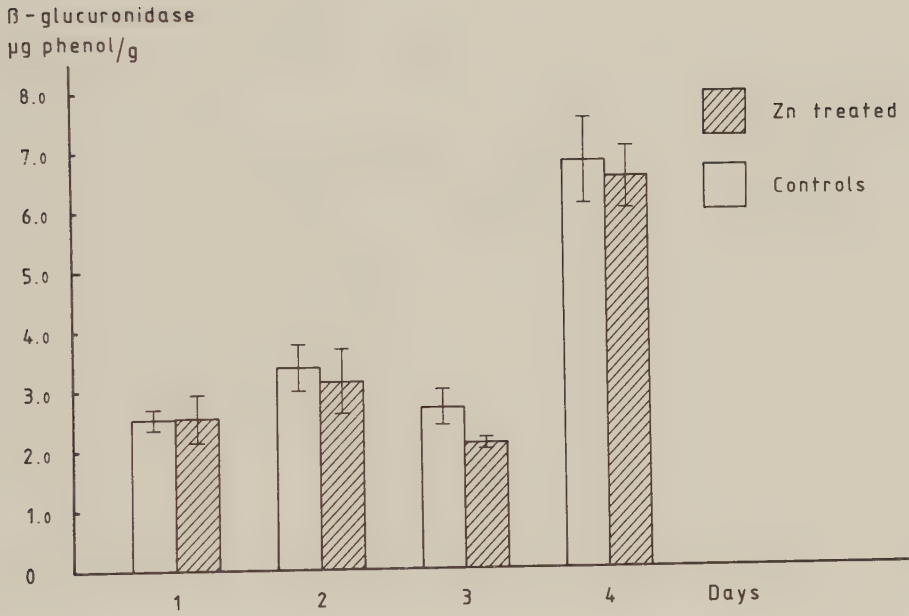


Fig. 3. Activity of β -glucuronidase in sponge homogenate after 1, 2, 3 and 4 days of implantation.

mainly in the mononuclear cells especially the macrophages. The activity increases during granulation tissue development. No difference can be visualized between the two groups.

Both fibroblasts and capillaries appeared histochemically almost enzymatically inert.

DISCUSSION

Granulation tissue was obtained by implanting polyvinyl sponges subcutaneously on the backs of rats (Woessner & Boucek, 1959). The sponge method was used as granulating wounds are not particularly suited for the study of biochemical changes during the early phase of wound healing. The configuration of the sponges influences the inflammatory reaction in the sponges (Pallin et al., 1975) with a prolonged inflammatory phase in cubic sponges. Therefore flat sponges were used in this investigation.

Histochemical techniques were used only for localisation of intracellular enzymes and quantification was based on biochemical determinations.

In this study the maximum granulocyte infiltration in granulation tissue is observed after 24 hours. This statement is based on the findings of highest activity of alkaline phosphatase at that time and histochemical location of alkaline phosphatase in granulocytes. The decreasing activity of alkaline phosphatase after the first day indicates that the granulocytes then disappear continuously from the granulation tissue. Raekallio (1970) in acutely injured skin observed histochemically an increase in alkaline phosphatase activity as early as eight hours after wounding. The

activity increased up to 32 hours. According to Manning & Dipasquale (1967) biochemically determined alkaline phosphatase shows two peaks during the healing of skin incisions in rats. The first peak occurs at 12 hours and is in accordance with the findings in our investigation. The second peak occurs at the tenth day, an interval not studied in our investigation.

The mononuclear cells increase during the observation time as indicated by the increasing activity of the lysosomal enzyme acid phosphatase localized in these cells. Other investigators (Raekallio, 1970) have observed increased acid phosphatase activity as early as 4 hours after injury, thereafter remaining unchanged during the first postoperative days.

The other lysosomal enzyme, β -glucuronidase, studied, does not increase until day 4. According to Pearse (1972) acid phosphatase and β -glucuronidase are not related in any constant manner but vary independently. Fishman et al. (1967) found activity of β -glucuronidase not only in the lysosomes but also in the endoplasmic reticulum. Fibroblasts have a well developed endoplasmic reticulum and may contain β -glucuronidase. At day 3 fibroblasts began to appear and increased in number to day 4. The increased activity of β -glucuronidase at day 4 may be explained by the appearance of fibroblasts and/or by maturation of monocytes to tissue macrophages.

The early inflammatory cell infiltration is not affected by zinc administration. However, a more rapid disappearance of granulocytes was observed in the zinc treated animals. Zinc administration does not seem to influence the mononuclear cell population.

In an earlier investigation (Tengrup et al., 1979) it was shown

that the amount of collagen is higher in granulation tissue at day 4 in animals given zinc. The higher amount found can be due to either increased synthesis or decreased break-down of collagen as the amount of collagen is the net result of collagen formation and break-down. The observed difference in granulocyte infiltration points to the possibility of a decreased collagen break-down depending on less granulocyte collagenase activity.

This investigation shows that zinc administration does influence the inflammatory reaction but does not clarify if zinc has an influence on the fibroblast activity as well. Studies of collagen synthesis in granulation tissue might help to clarify this question.

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V

Influence of Zinc on synthesis and accumulation of collagen in early granulation tissue

I. Tengrup, J. Ahonen and B. Zederfeldt

ABSTRACT

Chronic zinc deficiency causes delay of reparative processes. The rate of repair is normalized by zinc administration.

Acute lowering of serum zinc follows trauma. In a previous experimental study it was demonstrated that intramuscular zinc administration starting before trauma results in increased collagen accumulation in early granulation tissue.

In this study the rate of collagen synthesis was determined to find if the higher amount of collagen, found in early granulation tissue, could be explained by increased collagen synthesis. The rate of collagen synthesis was studied by determination of the incorporation of ^{14}C -L-proline into collagen *in vivo* and *in vitro*. No difference was found regarding collagen synthesis in granulation tissue from control and zinc treated animals.

Specific activity of collagen, a measure of newly synthesized collagen in relation to the total amount of collagen, was found to increase markedly during the observation time. Further, the specific activity was found to be lower in the zinc treated groups than in controls at day 4 and 5 indicating that after zinc administration there is more of earlier synthesized (not labelled) collagen present.

A possible explanation is that there is a concomitant synthesis and breakdown of collagen in granulation tissue and that the rate of breakdown is decreased by zinc administration.

Chronic zinc deficiency causes retardation of growth (Prasad, 1978) and delay of reparative processes (Hallböök & Lanner, 1972; Sandstead et al., 1970), probably as a consequence of impaired synthesis of nucleic acids and proteins, including collagen (Fernandez-Madrid, 1973; Sandstead & Rinaldi, 1969). The rate of repair is normalized by zinc administration (Hallböök & Lanner, 1972).

Acute lowering of serum zinc follows trauma (Hallmans, 1978; Sefton et al., 1974) and infections (Pekarek & Beisel, 1971). We have recently shown that the postoperative lowering of serum zinc is directly related to the magnitude of operative trauma (Tengrup & Samuelsson, 1977). The importance of acute lowering of serum zinc for reparative processes is not clear. However, in a previous experimental study (Tengrup et al., 1980) it was demonstrated that intramuscular zinc administration, starting before trauma, results in increased collagen accumulation in early granulation tissue. This indicates that acute lowering of serum zinc can be of importance for reparative processes.

The aim of the present study is to elucidate the mechanisms by which zinc administration causes increased collagen accumulation in early granulation tissue. Collagen synthesis in granulation tissue has been studied *in vivo* and *in vitro* by determination of the incorporation of ^{14}C -L-proline into collagen.

MATERIAL AND METHODS

Fifty white female Sprague-Dawley rats, weighing 180-200 g, were used. The rats were divided into groups according to Table I. All animals had free access to pellets (Astra-Ewos) and water during the experiment.

To obtain granulation tissue six polyvinyl sponges, each weighing 55-60 mg, were implanted subcutaneously on the backs of the rats according to Viljanto (1964).

Zinc treated rats were given 50 μ g zinc daily intramuscularly as zinc acetate solution with the first injection half an hour before the operation. The control animals received no zinc injections.

In the *in vivo* study of collagen synthesis 2.78 MBq ^{14}C -L-proline (10730 MBq/mmol, manufactured by NEN Chemicals Gmb, West Germany) was given intravenously into the tail of the rats 24 hours before removal of the sponges. In different groups the sponges were carefully removed 3, 4 and 5 days after implantation. The sponges were analysed for hydroxyproline content, and the radioactivity of ^{14}C -hydroxyproline (DPM ^{14}C -hypro per sponge) and

TABLE I. Number of animals at each interval.

	I n v i v o			I n v i t r o
	Day 3	Day 4	Day 5	Day 4
Controls	8	8	4	5
Zinc treated	8	7	6	4

total radioactivity (total DPM ^{14}C per sponge) were measured.

For the *in vitro* study 2 sponges from each animal were cut into 0.5 mm thick slices and incubated with 0.37 MBq ^{14}C -L-proline in a Krebs-Ringer solution for 150 minutes (Mobacken, 1974). The total radioactivity and the radioactivity of hydroxyproline were measured and the hydroxyproline content was determined.

The incorporation of ^{14}C -proline into proteins and collagen were determined according to Juva & Prockop (1966). Radioactivity was measured in a liquid scintillation counter (LKB Wallac 81000). At least 1000 cpm over background were registered. Counting efficiencies were determined by external standardization using a standard quenching diagram prepared from sponge sample with internal standardization. Hydroxyproline was determined according to Pikkarainen (1968) and was used for calculating specific activity of ^{14}C -hydroxyproline (DPM hypro/ μmol hypro).

Statistical methods

Comparisons between groups were carried out using Mann-Whitney rank sum test. Differences were considered significant when $p < 0.05$.

RESULTS

In vivo study

The amount of hydroxyproline was very low at day 3, but higher at day 4 and 5. At day 4 there is a higher amount of hydroxyproline in the zinc treated group than in the control group. No significant differences were registered between control and zinc treated animals at day 3 and 5 (Table II).

TABLE II. Collagen content (μmol hydroxyproline/sponge).

	Day 3	Day 4	Day 5
Controls	0.015 ± 0.001	0.021 ± 0.001	0.045 ± 0.007
Zinc treated	0.015 ± 0.001	0.028 ± 0.001	0.055 ± 0.005

The total radioactivity was the same in sponges removed at day 3 and 4 but was about twice as high in sponges removed at day 5. No differences were registered between control and zinc treated animals (Fig. 1).

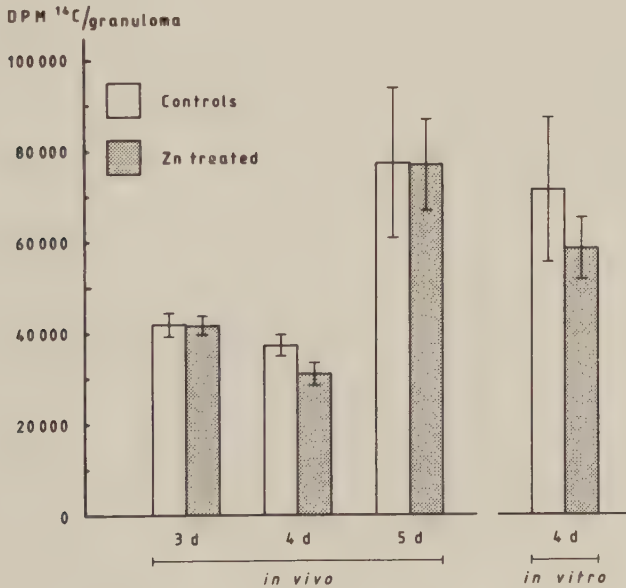


Fig. 1. Total radioactivity per granuloma.

The radioactivity of hydroxyproline per sponge is low at day 3, somewhat higher at day 4 and very much higher at day 5 (Fig. 2). There are no significant differences between control and zinc treated animals.

The specific activity of hydroxyproline is lower in the zinc treated animals at day 4 and 5 (Fig. 3).

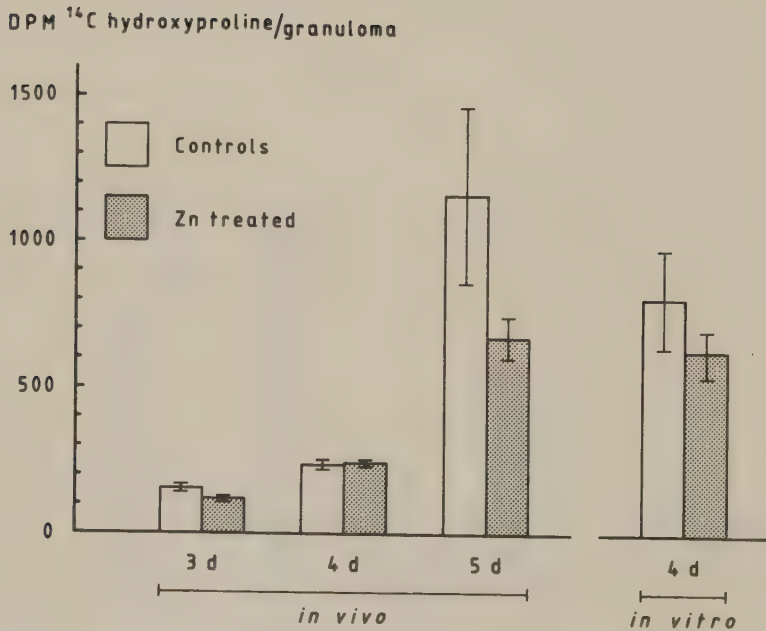


Fig. 2. Radioactivity of hydroxyproline per granuloma.

In vitro study

The total radioactivity is high after *in vitro* incorporation as is the radioactivity of hydroxyproline compared with the *in vivo* incorporation. There are no differences between control and zinc treated animal (Figs. 1 and 2).

The specific activity is lower in the zinc treated animals. The difference is more pronounced than after *in vivo* incorporation at the same interval (Fig. 3).

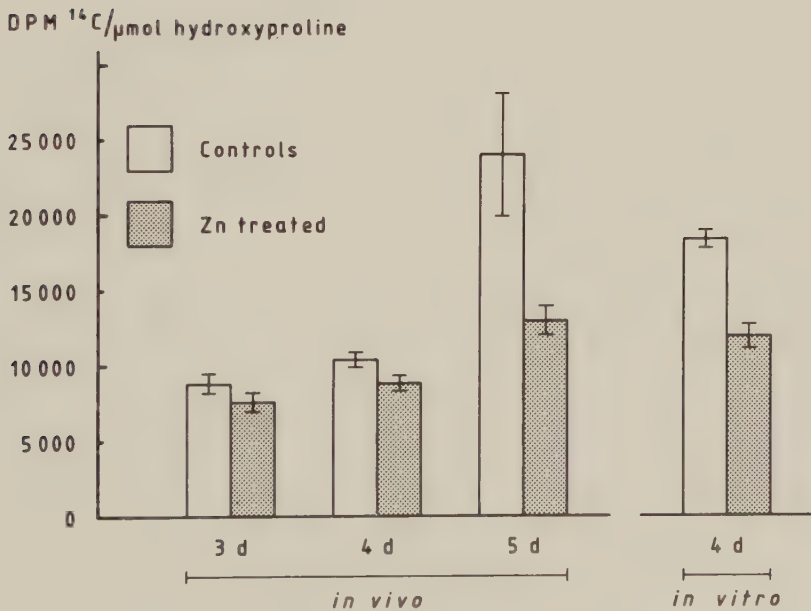


Fig. 3. Specific activity of hydroxyproline.

DISCUSSION

Granulation tissue was obtained by implanting flat polyvinyl sponges, a model earlier used and discussed (Tengrup et al., 1980). It has been shown that the tissue growing into such sponges is similar to normal granulation tissue both chemically and histologically (Viljanto & Kulonen, 1962; Woessner & Boucek, 1961). Flat sponges were used since cubic sponges demonstrate a more pronounced and prolonged inflammatory cell infiltration (Pallin et al., 1975).

The amounts of collagen in the sponges observed in this investigation are in accordance with earlier findings (Tengrup et al., 1980). At day 4 sponges from zinc treated animals contain more collagen than sponges from control animals.

The rate of collagen synthesis was determined to find if the higher amount of collagen found in granulation tissue after 4 days with zinc treatment, could be explained by increased synthesis of collagen. The rate of collagen synthesis can be studied by determination of the incorporation of ^{14}C -proline into collagen either *in vivo* or *in vitro*.

During collagen synthesis proline is incorporated into the peptide linkage of procollagen. A certain percentage of these incorporated proline residues is hydroxylated to hydroxyproline by a specific enzyme, prolyl hydroxylase (Prockop et al., 1979). Hydroxyproline as such cannot be incorporated into collagen. Therefore, determination of ^{14}C -hydroxyproline after administration of ^{14}C -proline is a measure of the rate of collagen synthesis.

Both the *in vivo* and the *in vitro* method to study the rate of collagen synthesis have certain limitations. The *in vivo* pulse

labelling technique (Jiborn et al., 1980) can give erroneous results regarding the rate of collagen synthesis if marked changes occur in the size of the amino acid pools. The *in vitro* technique requires slicing of the sponges which can result in a loss of tissue components (Roberts, 1977). This may occur especially when early granulation tissue is studied as in the present investigation. For this study we have chosen the *in vivo* pulse labelling technique but a control study using *in vitro* incorporation was also made. The results obtained with the two methods were in accordance.

In this investigation no difference was found regarding collagen synthesis (registered as DPM ^{14}C -hydroxyproline) in granulation tissue from control and zinc treated animals. The increased amount of collagen found at day 4 can thus not be explained by increased collagen synthesis.

Further information about the mechanism for the influence of zinc on collagen accumulation can be obtained by calculation of the specific activity of collagen. This is a measure of newly synthesized collagen in relation to the total amount of collagen in the specimen. Specific activity was found to increase markedly from day 3 to day 5 which is a consequence of increasing rate of collagen synthesis during this period. Further, the specific activity was found to be lower in the zinc treated groups than in controls at day 4 and 5. This finding indicates that after zinc administration there is more of earlier synthesized (not labelled) collagen present in the granulation tissue.

A possible explanation for these findings is that in granulation tissue there is a concomitant synthesis and breakdown of col-

lagen and that the rate of breakdown is decreased by zinc administration.

In somewhat older granulation tissue, decreasing collagen content despite persisting high rate of collagen synthesis indicates concomitant breakdown of collagen (Madden, 1970). Further, Peacock (1967) was able to show collagenolytic activity in granulation tissue and has speculated that there is as well a rapid breakdown as a synthesis of collagen in granulation tissue.

Decreased breakdown of collagen in early granulation tissue after zinc administration could be the consequence of an altered inflammatory reaction in the granulation tissue. In a previous study (Tengrup et al., 1980) it has been shown that polymorphonuclear leucocytes disappear more rapidly from granulation tissue after zinc administration. The polymorphonuclear leucocytes, which are the most prominent cells in early granulation tissue, contain several proteolytic enzymes, some of which are also collagenolytic, e.g. collagenase, elastase and acid cathepsins. An earlier disappearance of this source of collagenolytic enzymes could mean decrease in the rate of breakdown of collagen.

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